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ROLE OF NSAIDS DRUG AS ANTI-INFLAMMATORY AGENT

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ABSTRACT

Inflammation is body's natural Defence mechanism to neutralize an injury or presence of a foreign substance. In the event of a perceived invasion a foreign substance, the white blood cells secrete chemicals into the target area that may result in redness, warmth, swelling and pain. In the case of autoimmune diseases such as rheumatoid arthritis, the immune system is provoked even in the absence of a foreign body rendering unpleasant symptoms of inflammation. The development of synthetic drugs to manage inflammation and pain has been the major goal of many drug discovery programs around the world. Non-steroidal Anti-Inflammatory Drugs (NSAIDs) drugs are widely used therapeutics for the treatment of pain and inflammation. They showed great promise due to their synthetic viability and affordability. Traditional NSAIDs, such as Aspirin, Naproxen, Piroxicam, Ibuprofen, Diclofenac, etc., inhibit both COX-1 and COX-2, which accounts for NSAIDs anti-inflammatory effects as well as their notorious side effects of GI toxicity and blood thinning. Thus, inhibition of COX-2 over COX-1 should be useful for treatment of inflammation without incurring the side effects associated with inhibition of COX-1. The mechanism by which these drugs alleviate pain and inflammation has been found to be inhibition of prostaglandin synthase (Cyclooxygenase, COX), that is responsible for formation of prostanoids, including thromboxane and prostaglandins such as prostacyclin. The COX exists as two isoforms that is COX-1 and COX-2. COX-1 is responsible for the maintenance of mucosal integrity of gastrointestinal tract, while COX-2 plays a pivotal role in the process of inflammation. A poor selectivity for COX-2 was shown to result in mucosal damage, nephrotoxicity and undesirable cardiovascular side effects due to its COX-1 inhibition. Many

drugs that were introduced into the market as anti-inflammatory agents (Rofecoxib and Valdecoxib) eventually had to be withdrawn due to their unbearable side effects. Research groups across the globe have been in the quest for a potent and COX-2 inhibitor. Recently, many groups reported that N-acyl hydrazones (NAH) pharmacophore containing compounds as new class of anti-inflammatory agents by targeting COX-2 enzyme. These studies inspired us to design new hybrid NAH containing COX-2 inhibitors as anti-inflammatory agents. In the current study, we have designed set of NAH hybrid compounds and confirmed their binding modes using docking methods. Further, these compounds were synthesized and biologically tested as COX-2 inhibitors.
Key words: Inflammation, COX-2 inhibitors, synthetic drugs

INTRODUCTION

The primary focus of many researchers remains the creation of novel, potent chemical architectures with biological activities such as anti-inflammatory, analgesic, antibacterial, antifungal, antiviral, antihypertensive, antidepressant, anticancer, antiplatelet, antimalarial, and anticonvulsant, among others. Due to their numerous biological functions, hydrazone derivatives containing the azomethine -NHN=CH- group, among others, receive a lot of attention. An important class of compounds with a wide range of pharmacological activities includes hydrazone and its derivatives with the azomethine -NHN=CH- group. The potential pharmacological properties of a number of hydrazone derivatives, which have been synthesized and reported in the literature, include anti-inflammatory, antibacterial, analgesic, antifungal, antihypertensive, antiplatelet, anticancer, antimalarial, antidepressant, anticonvulsant, and antiviral effects, among others. When they are modified to other functional groups, they produce pharmacologically active molecules in addition to their extended biological properties.

Acyl hydrazones belong to a very ancient class of substituted hydrazones: Since the discovery of the first examples of N-acyl hydrazones in 1850, researchers all over the world have looked into a wide range of novel N-unsubstituted, mono-, and disubstituted acyl hydrazines. Acyl hydrazones with nitrogen in them have been shown to be versatile chemical reactions that can be used as precursors and intermediates to make important organic molecules like polymers, pharmaceuticals, and heterocycles.

Inflammation

Immune cells, blood vessels, and molecular mediators are all involved in inflammation, which is the body's limited response to infections and tissue injury[1]. The inducing stimulus may be endogenous, such as molecules released by damaged tissues, sodium urate crystals, oxidized lipoproteins, or degradation products of the extracellular matrix[2], or exogenous,

such as microorganisms, allergens, or foreign particles. The inflammation-affected areas of the body then become hot, red, protruding, and painful. Enzyme activation, mediator release, extravasation of fluid, cell migration, pathogen elimination, localization of damage, elimination of the early cause of cell injury, clearance of necrotic cells, tissue damaged from the initial insult, and the inflammatory process are just a few of the many processes that occur during inflammation[3].

There are three distinct phases to the inflammatory response[4].

(A) A critical phase:

Several clinical signs, including erythema, edema, hyperalagesia, and loss of function, are associated with it. The increased vascular permeability causes fluids to leak from the blood vessels into the interstitial space[5].

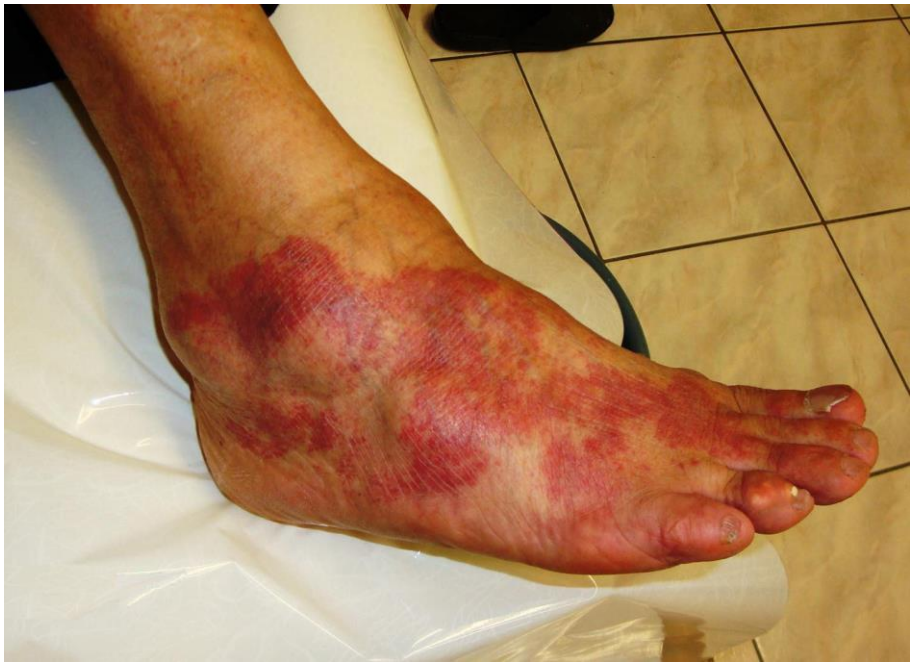


Figure 1. Inflammation

The cardinal signs of inflammation include: pain, heat, redness, swelling, and loss of function. Some of these indicators can be seen here due to an allergic reaction.

(Courtesy to Wikipedia)

(B) An intermediate stage:

It is characterized by the blood's infiltration of phagocytic leukocytes into the tissue.

(C) A phase of persistent proliferation:

Granuloma formation is its defining feature.

The immune system's adaptive and innate cells work together to coordinate the inflammatory response. The innate branch includes monocytes, macrophages, dendritic cells, mast cells, and other cells that are targeted against common pathogen antigens[6]. The adaptive branch includes T- and B-cells that can induce long-term memory of encountered pathogens. There are four parts to a typical inflammatory response: inflammatory inducers, sensors that detect them, inflammatory mediators, and effectors, or target tissues.

These sensors, mediators, and effectors are chosen so that the appropriate inflammatory response is induced based on the type of infection. Traditionally, inflammation develops quickly, stays localized, and disappears without lasting effects after pathogens are eradicated. However, the associated inflammatory risk factors can result in malignant or even fatal outcomes in some instances of severe infection or trauma with defective regulation. These inflammatory processes are the cause of numerous serious pathological conditions like cancer, metabolic syndrome, diabetes, atopy, atherosclerosis, cardiovascular disease, and some neurodegenerative diseases[7]. This made us realize the significance of studying inflammatory mechanisms and the need to develop effective anti-inflammatory treatments. Anti-inflammatory medications have been developed to prevent the production of pathologically produced prostaglandins[8], as the name suggests. This prevents inflammatory processes from occurring. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are examples of these medications.

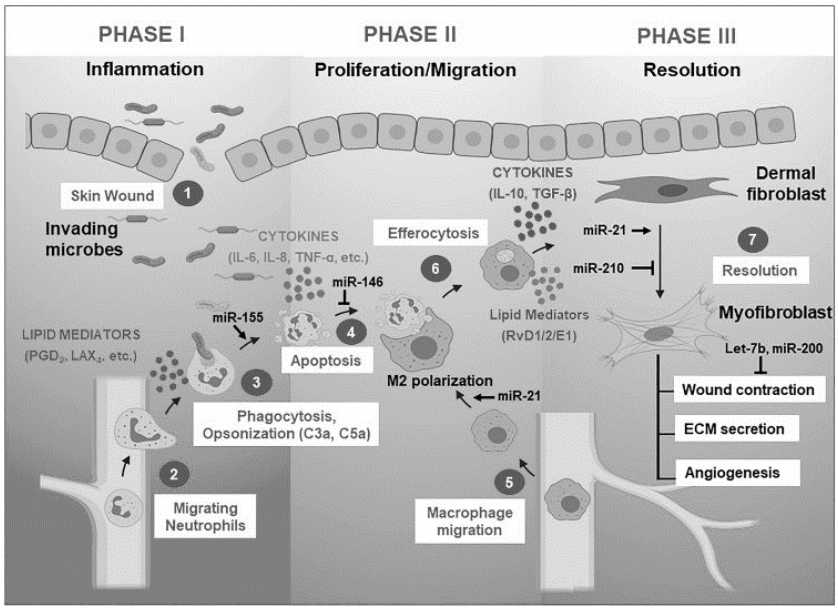


Figure 2. Phases of Inflammation (Courtesy to Wikipedia)

The history of NSAIDs:

Salicylate-rich plants have been used to treat a variety of diseases for thousands of years[9]. With no real understanding of the pharmacology of the substances being used, the medicinal value of the willow leaves (reducing the fever and pain during childbirth), the juices of the poplar tree (treating eye diseases), the dried leaves of myrtle (relieving rheumatic pains from the womb), and the other sources of salicylates continued to be documented throughout the Middle Ages and in all parts of the world. Stone and others' clinical trials with willow bark prompted the isolation and purification of the active principle in willow bark for further research[10]. However, in 1829, Leroux isolated salicin, a glycoside with potent antipyretic properties, the active ingredient in willow bark. Salicin hydrolysis yields glucose and salicylic alcohol, which can be converted into salicylic acid in vivo or through chemical manipulation. The discovery of salicin's antipyretic, anti-inflammatory, and analgesic properties in 1874 set the stage for the long-term use of salicylates[11] The synthesis of salicylic acid in practice was clarified by Kolbe and Lautemann[12]. The treatment of rheumatoid fever[13] is the first application of salicylic acid. Due to its greater water solubility, lower toxicity, and relatively simple manufacturing, sodium salicylate emerged as the preferred compound following the discovery of salicin and salicylic acid's therapeutic efficacy against rheumatic fever. By acetylating the hydroxyl group on salicylic acid's benzene ring, Hoffman produced acetylsalicylic acid in 1897. After conducting extensive pharmacological and toxicological tests, his colleague Dreser reported that the new compound was immediately converted into salicylic acid upon absorption into the body. Additionally, it had a more pleasant flavor and was more easily tolerated by the gastric mucosa[14].

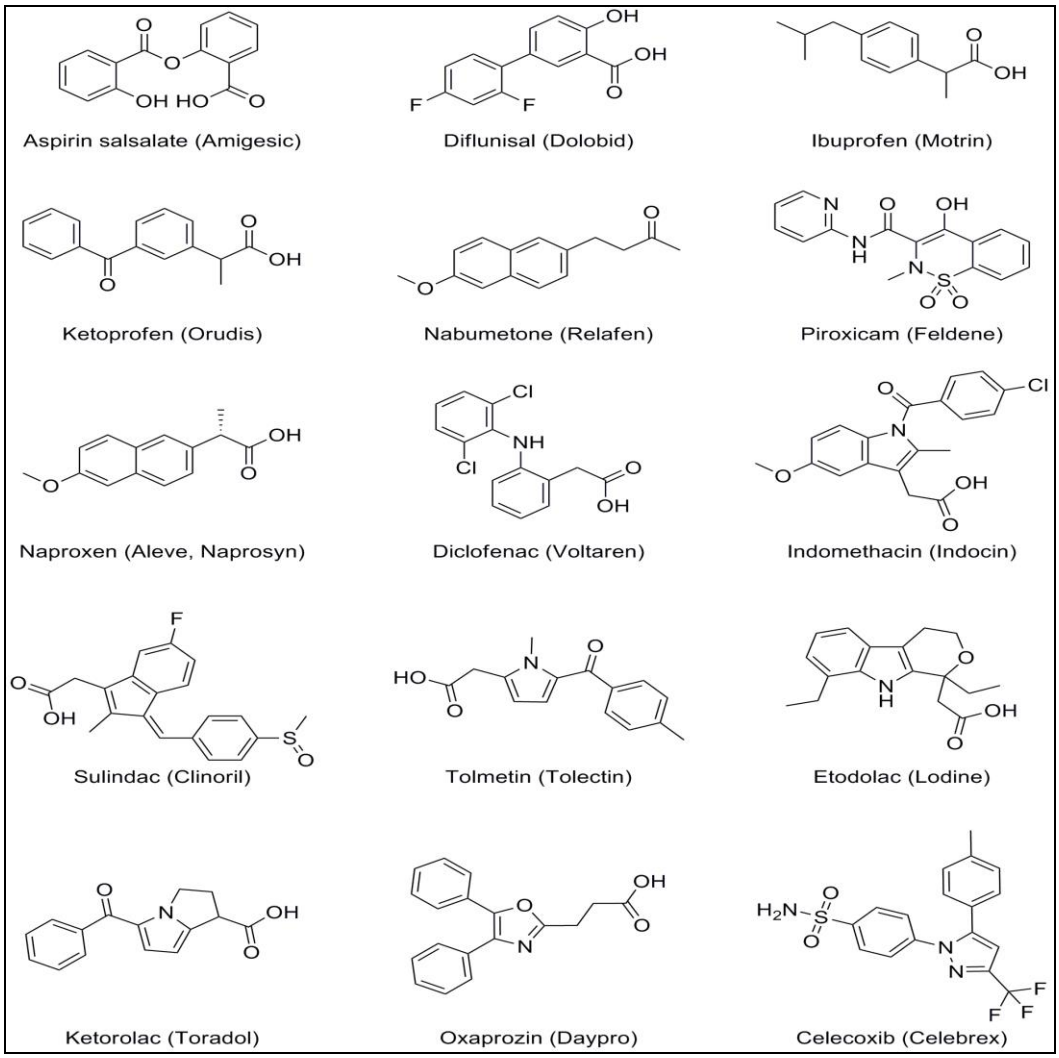
In the treatment of inflammation, mild to moderate pain, and fever[15], non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications. The medications are widely available, affordable, and simple to obtain. In the late 1950s, there was a push for non-steroidal anti-inflammatory drug research with the goal of separating corticosteroids' serious side effects from their anti-inflammatory properties. Phenylbutazone, the first non-steroidal anti-inflammatory drug, was introduced to the market in 1953, but there were soon additional reports of side effects[16]. After a study of their fundamental mechanisms revealed their toxicity, they gained attention. Clinical trials and observational studies have strengthened this relationship, which led to the findings.

Aspirin and indomethacin were found to be able to inhibit prostaglandin formation at therapeutic concentrations[17] in 1971, despite the development of numerous NSAIDs. Prostaglandins influence important cellular and vascular changes during inflammation, suggesting that stopping their biosynthesis would reduce pain, fever, and inflammation[18]. Fenamates and diflunisal, for example, were developed as a result of these findings.

Diclofenac[19], another potent NSAID, is an analog of fenamates. Diflunisal, on the other hand, is a new salicylic acid analogue that is less stomach-irritating than most nonsteroidal anti-inflammatory drugs (NSAIDs)[20] and approximately four times as effective as aspirin as an analgesic and anti-inflammatory agent.

The following are some of the most widely used NSAIDs:

NSAIDs are used to treat headaches, arthritis, ankylosing spondylitis, sports injuries, menstrual cramps, and other conditions[21]. The inhibition of COX enzymes, which prevents the conversion of arachidonic acid into prostaglandins, prostacyclins, and thromboxanes, is the basis for the anti-inflammatory activity of NSAIDs. Athletes frequently make use of them as ergogenic aids and for treating injuries. There are a variety of side effects associated with NSAIDs, varying in frequency[22].



Examples of NSAIDs

Nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache, and drowsiness are the most frequent adverse effects. Kidney failure, liver failure, ulcers, and prolonged bleeding following injury or surgery[23] are the persistent side effects. The most serious secondary effects on the stomach related track include gastroduodenal holes and gigantic hemorrhages. Long-term use of NSAIDs causes inflammation in the small intestine, which results in a loss of blood and proteins[24]. Aspirin prevents strokes and heart attacks in people who are at high risk for them[25] by inhibiting blood clotting for a prolonged period of time. Ketorolac is a powerful nonsteroidal anti-inflammatory drug (NSAID) used to treat moderately severe acute pain for short periods of time26. Celecoxib is used to treat familial adenomatous polyposis and helps stop colon polyps from growing and developing[27].

The Cyclooxygenase's Role in Inflammation:

An enzyme in the class of isozymes is cycloxygenase (COX), also known as prostaglandin-endoperoxide synthase (PTGS). Prostanoids like thromboxane and prostaglandins like prostasyclin and prostaglandin E are made by it. This is the enzyme that initiates inflammation and is a crucial regulator in the biosynthesis of prostaglandins[28]. The prostaglandins are arachidonic acid-derived lipid autacoids that are produced by the cyclooxygenase pathway. The pharmaceutical inhibition of the COX enzyme can alleviate pain and inflammation symptoms[29].

Culinary mushrooms, a variety of flavonoids, fish oils, hyperforin, and calcitriol (vitamin D) naturally inhibit the COX enzymes. Additionally, NSAIDs attempt to exert their effects by inhibiting COX30. Arachidonic acid is first converted into prostaglandins, thromboxane, and prostacyclin by inhibiting the COX enzyme. They also have negative effects on the human body, such as inhibiting mitochondrial oxidative phosphorylation, in addition to inhibiting COX. Additionally, they stop the lipoxygenase pathway, which causes leukotrienes to be made.

The mechanisms by which NSAIDs inhibit COX-1 and COX-2 vary, but there are exceptions. Nimesulide, for example, is a weak competitive inhibitor of COX-1 but a potent time-dependent inhibitor of COX-2. Celecoxib, on the other hand, exhibits slow competitive binding but binds irreversibly at higher concentrations[31].

The three types are as follows:

Ibuprofen, Piroxicam, and Mefenamic acid are examples of COX-1 and COX-2 reversible binders in Category 1; whereas COX-1 and COX-2 are reversibly bound in a time-dependent

manner by Category 2 (such as Diclofenac, Flurbiprofen, and Indomethacin), which is slower and lower affinity.

Category 3 involves the rapid reversible binding of COX-1 and COX-2, such as aspirin, followed by covalent modification [28,32].

Ibuprofen, for example, inhibits arachidonic acid binding in a competitive manner and rapidly separates from the COX active site. In contrast, the slow, time-dependently binding NSAIDs, like flurbiprofen, initially compete poorly with arachidonic acid but eventually bind tightly. Both ibuprofen and flurbiprofen embrace a comparative compliance in the dynamic site, and it has been recommended that the motor distinctions between classification 1 and 2 NSAIDs is the speed and productivity by which they get entrance through the thin choking in the COX channel made by Arg120, Tyr355 and Glu52422. Since the COX-2 inhibitors lack a carboxyl group, the charged interaction with Arg120 is not necessary for their binding to the COX active site. Instead, as the sulfonamide moiety slowly orients itself within the hydrophobic side pocket[33], the larger methylsulfonylphenyl derivatives block the COX-2 channel over time. Due to the lack of access to the side pocket, it is believed that COX-2 inhibitors simply inhibit COX-1 through competitive inhibition. Due to its weak ionic bond with Arg120, aspirin is unique among NSAIDs because it orients itself within the COX active site. This makes it easier to trans acetylate Ser530, which completely blocks COX activity. It is evident that the kinetic diversity of the COX inhibition mechanism renders simple comparisons between NSAIDs of questionable validity, making it difficult to extrapolate COX inhibition to cause–effect relationships.

COX enzyme types include:

The expression pattern of the cyclooxygenase enzyme's two related isoforms primarily differs. COX-1 (PTGS-1) is expressed in the majority of tissues, but COX-2 (PTGS-2) is typically absent. The regulation of prostaglandin biosynthesis and prostaglandin-dependent effects on blood flow and tissue integrity require COX-1, a constitutive enzyme34. Numerous physiologic stimuli in the tissues induce COX-2. While cyclooxygenase-2 (COX-2) is responsible for the elevated production of prostanoids at the site of disease and inflammation, cyclooxygenase-1 (COX-1) is responsible for the physiological production of prostanoids. Surprisingly, the mouse's gastrointestinal system was unaffected by COX-1 disruption35. Mice deficient in COX-2 have abnormal reproductive functions and kidney development36. A "house-keeping enzyme," COX-1 regulates normal cellular processes like platelet aggregation, kidney function, gastric cytoprotection, and vascular homeostasis37. Some tissues, like the brain, kidney, and bone, have constant levels of COX-2 expression, while other locations have higher levels of COX-2 expression when there is inflammation. The two compounds share 60% homology in their amino corrosive sequence38. However, COX-2 has

a larger and more flexible substrate channel than COX-1 does, and COX-2 also has a larger space at the site where inhibition binds. This structural difference between COX-1 and COX-2 has made it possible to develop a selective COX-2 inhibitor39. However, the involved conformations for the substrate binding sites and catalytic regions are slightly different. These findings led to the following: a) The non-selective inhibitors have access to the binding channels of both isoforms; b) The larger COX-1 residues, Ile434, His513, and Ile532, prevent the COX-2 inhibitors' access to the bulky side chains40. Studies, have demonstrated the way that glucocorticoids can smother the development of proteins engaged with aggravation (bringing about their job as mitigating compounds).

Aside from that, glucocorticoids work by activating a group of enzymes called lipocortins to further reduce inflammation. It has been discovered that lipocortins inhibit or reduce the activity of phospholipase A2, a crucial enzyme in the process of releasing arachidonic acid from the cell membrane, where it is typically incorporated. Arachidonic acid is converted into substances like prostaglandins, which mediate inflammation, when the cell is attacked by foreign substances. This acid is released from the cell membrane. The COX-2 isozyme synthesizes inflammatory prostaglandins using free arachidonic acid. Phospholipase A2 must be activated before arachidonic acid can be released. The phospholipase A2 activity is inhibited by the lipocortins, as previously mentioned. Glucocorticoids contribute to their effectiveness as significant anti-inflammatory agents by activating lipocortins and inhibiting phospholipase A2 by inhibiting the release of arachidonic acid and prostaglandin synthesis in the cell. Inflammation is reduced and damage caused by chronic inflammation is reduced because fewer inflammatory prostaglandins are synthesized.

COX-2 inhibitors as drugs that reduce inflammation:

Since COX-2 is an inductive enzyme that is found in inflammatory cells, it is thought to be a good target for reducing inflammation. The idea that selective COX-2 inhibitors are a second generation of nonsteroidal anti-inflammatory drugs (NSAIDs) without the undesirable side effects that lead to gastrointestinal (GI) damage[41] was presented by the discovery of the second cyclooxygenase enzyme (COX-2) at the beginning of the 1990s. Additionally, the relatively small difference in size between the active centers of COX-1 and COX-2 has been used by researchers to develop selective COX-2 inhibitors such as celecoxib[42], The constitutive COX-1 isozyme appears to be necessary for maintaining normal physiological functions like gastric protection and vascular homeostasis because it is produced in a variety of tissues. On the other hand, the COX-2 isozyme is induced by pro-inflammatory and mutagenic stimuli, indicating that it is involved in inflammatory processes. As a result, when compared to conventional nonsteroidal anti-inflammatory drugs (NSAIDs), selective inhibition of COX-2 over COX-1 is beneficial for the treatment of inflammation and disorders that are associated with inflammation. COX-2 has been linked to rheumatoid

arthritis and osteoarthritis, as well as colon cancer and angiogenesis[45,46]. Some people who take NSAIDs have a lower risk of developing Alzheimer's disease, according to recent research. Therefore, without causing damage to the gastrointestinal tract, ongoing treatment with selective COX-2 inhibitors might slow the progression of Alzheimer's disease.

The objectives of the study are:

1. To design, synthesis and the biological evaluation of anti-inflammatory activities of *N*-acylhydrazone linked isoxazoles using rational drug design approach.

2. To calculate the binding affinities of newly designed compounds and to compare with known anti-inflammatory NAH and cyclooxygenase-2 (COX-2) inhibitors (coxibs) at the active sites of cyclooxygenase-1 (COX-1) and COX-2.

3. Based on the findings to synthesize the compounds and to subject them to *in vitro*, *in vivo* anti-inflammatory studies.

4. To evaluate for antioxidant activity and to compare with ascorbic acid.

5. To screen these compounds for anti-bacterial activities against gram *+ve* and *–ve* and compare them standards Ciprofloxacin and Flucloxacillin.

6. To design, synthesis and the biological evaluation of anti-inflammatory activities of *N*-phenyl sulfonamide linked *N*-acylhydrazones (NPS-NAH) using molecular hybridization approach.

7. To validate hybrid compounds with theoretical studies.

8. To conduct an effective *in vivo* anti-inflammatory profiling of potent and selective COX-2 inhibitors and to compare with known COX-2 inhibitors.

9. To test these compounds for antioxidant activity

10. To screen these compounds were screen for antibacterial activity with gram *+ve* and *–ve* and compare with reference standards: Ciprofloxacin and Norfloxacin

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